

What is claimed is

1. A method of screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* comprising:

cloning a nucleic acid fragment which encodes a peptide DBD of a transcription factor into an expression vector to yield a construct (1) such that the DBD may be expressed in a bio-active form and bind a corresponding DNA regulatory sequence binding site in a heterologous host cell,

fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule into construct 1, in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),

cloning an sFv library into a DNA construct to yield a construct (3) such that a single chain monoclonal antibody may be expressed in bio-active form and bind a corresponding antigen in a heterologous host cell,

fusing a nucleic acid fragment which encodes a trans-activation peptide into construct 3, in the same translation reading frame of the nucleic acid fragment which encodes the single chain monoclonal antibody, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell,

providing a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2,

introducing constructs 2 and 4 into the heterologous host cell harboring a detectable gene

under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, such that both constructs may be expressed, and

identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene.

2. The method of Claim 1 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, to yield a modified construct (5) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

3. The method of Claim 1 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, and deleting the TA, to yield a modified construct (6) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

4. The method of claim 2 wherein the transcriptional associated biomolecule is selected from the group consisting essentially of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

5. The method of claim 4 wherein the transcriptional associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase C γ -PLC γ , phosphatidylinositol 3-kinase-PI3K, Syp, mitogen activated protein kinase-MAPK, jun kinase-JNK, androgen receptor (AR), thyroid hormone receptor (TR), glucocorticoid receptor (GR), ATF-1, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM τ .

6. The method of claim 3 wherein the transcriptional associated biomolecule is selected from the group consisting essentially of a transcription factor, ligand, hormone, nuclear hormone receptor, DNA binding domain of nuclear hormone receptor, tumor associated protein, protein kinase, protein phosphatase, GTP binding protein, adaptor protein, secondary messenger of an intracellular signaling molecule, and a protein derived from an etiological agent.

7. The method of claim 6 wherein the transcriptional associated biomolecule is selected from the group consisting of Ras, Grb2, phospholipase C γ -PLC γ , phosphatidylinositol 3-kinase-PI3K, Syp, mitogen activated protein kinase-MAPK, jun kinase-JNK, androgen receptor (AR), thyroid hormone receptor (TR), glucocorticoid receptor (GR), ATF-1, ATF-2, ATF-3, ATF-4, ATF-6, CREB and CREM τ .

8. A method of screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* comprising:

providing an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),

providing a DNA construct (3) which encodes a trans-activation peptide and comprises a

cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell,

providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and

identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene.

9. A single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* isolated by a method comprising:

cloning a nucleic acid fragment which encodes a peptide DBD of a transcription factor into an expression vector to yield a construct (1) such that the DBD may be expressed in a bio-active form and bind a corresponding DNA regulatory sequence binding site in a heterologous host cell,

fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule into construct 1, in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),

cloning an sFv library into a DNA construct to yield a construct (3) such that a single chain monoclonal antibody may be expressed in bio-active form and bind a corresponding antigen in a

heterologous host cell,

fusing a nucleic acid fragment which encodes a trans-activation peptide into construct 3, in the same translation reading frame of the nucleic acid fragment which encodes the single chain monoclonal antibody, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell,

providing a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2,

introducing constructs 2 and 4 into the heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, such that both constructs may be expressed,

identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene, and

isolating the single chain monoclonal antibody fusion reagent capable of binding the transcriptional associated biomolecule *in vivo*.

10. The single chain monoclonal antibody fusion reagent of Claim 9 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, to yield a modified construct (5) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the

corresponding antigen in a heterologous host cell.

11. The single chain monoclonal antibody fusion reagent of Claim 9 further comprising fusing at least one nucleic acid fragment which encodes an intracellular targeting signal in the same translation reading frame to the nucleic acid fragment which encodes the single chain monoclonal antibody in construct 4, and deleting the TA, to yield a modified construct (6) such that a resulting single chain monoclonal antibody fusion reagent may be expressed in bio-active form and bind the corresponding antigen in a heterologous host cell.

12. A single chain monoclonal antibody fusion reagent according to Claim 9 which is capable of regulating transcription *in vivo*.

13. A single chain monoclonal antibody fusion reagent according to Claim 10 which is capable of regulating transcription *in vivo*.

14. A single chain monoclonal antibody fusion reagent according to Claim 11 which is capable of regulating transcription *in vivo*.

15. A therapeutic method for regulating the transcription of a gene *in vivo* comprising administering an effective amount of a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* identified by a method comprising:

providing an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),

providing a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell,

providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and

identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene.

16. A therapeutic method for regulating the transcription of a gene *in vivo* according to Claim 15 wherein the single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* comprises at least one intracellular targeting signal fused to the single chain monoclonal antibody.

17. A method of screening a plurality of compounds for specific binding affinity with a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* identified by a method comprising:

providing an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the

nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2),

providing a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell,

providing a heterologous host cell, harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and

identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene, and

screening a plurality of compounds comprising the steps of:

providing a plurality of compounds,

combining the single chain monoclonal antibody fusion reagent with each of a plurality of compounds for a time sufficient to allow binding under suitable conditions; and

detecting binding of said single chain monoclonal antibody fusion reagent to each of the plurality of compounds, thereby identifying the compounds which specifically bind said single chain monoclonal antibody fusion reagent.

18. A method for diagnosing a physiological disorder manifested by abnormal levels of a transcription associated biomolecule, said method comprising:

contacting a biological sample with a labelled single chain monoclonal antibody fusion reagent or a portion thereof according to Claim 9 whereby said antibody reagent binds to said transcription associated biomolecule to form a complex,

separating unbound labelled antibody reagent from said complex,

measuring the amount of bound labelled antibody reagent in said complex; and,

comparing the quantity of labelled antibody reagent in said biological sample to the quantity of labelled antibody reagent which binds to normal biological samples under identical conditions.

19. A pVP16Zeo library expression vector (ATCC deposit # ____) for the construction and screening of single chain monoclonal antibody fusion reagent libraries, comprising zeocin selection to facilitate the isolation and production of single chain monoclonal antibody fusion reagents in yeast and *E.coli*.

20. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo*; comprising in a container:

an expression construct (1) which encodes a peptide DBD of a transcription factor and comprises a cloning site for fusing a nucleic acid fragment which encodes an antigenic portion of a transcriptional associated biomolecule in the same translation reading frame of the nucleic acid fragment which encodes the DBD of a transcription factor, to yield a construct (2), and

a DNA construct (3) which encodes a trans-activation peptide and comprises a cloning site for fusing an sFv library in the same translation reading frame of the trans-activation peptide, to yield a construct (4) such that a resulting chimeric sFv/trans-activation peptide may be expressed in bio-active form and bind a transcriptional associated biomolecule in a heterologous host cell, and

a heterologous host cell harboring a detectable gene under transcriptional control of the DNA regulatory sequence binding site corresponding to the DBD encoded by construct 2, for introducing constructs 2 and 4 into the heterologous host cell, such that both constructs may be expressed, and

a means for identifying a DNA construct 4 which encodes a single chain monoclonal antibody reagent capable of binding the transcriptional associated biomolecule *in vivo* by selecting for expression of the detectable gene.

21. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* according to Claim 20 wherein DNA construct 3 is pVP16Zeo (ATCC deposit # ____).

22. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* according to Claim 21 wherein primers are provided for human sFv library construction.

23. A kit for screening a DNA construct library for a single chain monoclonal antibody fusion reagent capable of binding a transcriptional associated biomolecule *in vivo* according to Claim 22 wherein primers selected from the group consisting essentially of (SEQ ID NOs: 3 - 86) are provided for human sFv library construction.